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New 5,6-Dihydro Pyrimidine Derivatives, Synthesis, Characterization and Antibacterial Activity

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ABSTRACT— In this study, five Schiff base compounds were prepared by the reaction between one mole of benzil with one mole of the below five types of amines: (aniline, 1 – naphthalamine, 4-nitroaniline, 4-chloroaniline, and 4-aminobenzoic acid). Such products were transferred into chalcone by reacting them with acetone. The resulted compounds were reacted with urea to prepare five derivatives of imine – based 5,6 – dihydropyrimidin – 2(1*H*) – one. Such resulted compounds were traced via spectrometry [MASS, (^{1}H, ^{13}C NMR) and FTIR]. The results conformed with the expected compounds. The biological activity of the synthesized compounds was then tested against two types of bacteria (*Staphylococcus aureus* and *E-coli*). It has been approved accordingly that such prepared compounds have a moderate effect against bacteria.

KEYWORDS: Antibacterial activity, Chalcone, Pyrimidine, Schiff base.

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1. INTRODUCTION

Imine compounds, that is, Schiff bases are important compounds in biochemistry that many derivatives of Schiff basis have been used in various fields including pharmacy and industry [1]. With regards to the pharmacy, Schiff bases have been used as one of the materials used in the preparation of antidepressant, anti-cancer and anti-inflammatory drugs [2]. As to industry, the Schiff basis has been used in the field of polymer synthesis [3], [4]. They have also been used as an additive to polymers, where they produce additives that prevent photo-oxidation and thermal oxidation [5], [6]. Using Schiff base, many compounds have been prepared, including heterocyclic compounds, which are of great importance in many fields, including industrial and pharmaceutical fields [7]. Other compounds that are no less important than Schiff bases are α, β-unsaturated ketone compound is deemed as one of the important compounds used in the field of antioxidants [8]. It is used as a starting material in the synthesis of antioxidant compounds [9], [10]. Its significant importance is indicated by its role in preparing heterocyclic compounds [11]. Heterocyclic compounds are known to be involved in the synthesis of many biological molecules, including enzymes and hormones [12]. One of the most important heterocyclic compounds that can be prepared from α, β-unsaturated ketone compound is pyrimidine which is deemed as one of the most important heterocyclic compounds because it is involved in the creation of RNA and DNA [13].

As to the biological aspect, it is one of the important compounds used in many reactions which lead to the preparation of many antioxidant drugs, especially colon cancer drugs which act to eliminate free radicals [14].

Based on the foregoing as to the importance of the said compounds, new compounds have been prepared out of 5,6- dihydropyrimidin – 2(1*H*) –one.

2. STUDY DESIGN

(^{1}H,^{13}CNMR, IR and MASS) spectroscopy was used to prove the compositions of the synthesized compounds.

The following equipment was used to identify the synthesized compounds:

Shimadzu to record FTIR spectra, Bruker to record NMR spectra, Shimadzu QP to record Mass spectra, melting point was measured by Electrothermal IA device.

The materials used were of high purity. Glacial acetic acid 99%. (Benzil, thiourea, urea, p-aminobenzoic and absolute ethanol) 98%. Acetone 97%. Sodium hydroxide 96%. (p-Chloroaniline, p-nitroaniline and 1-naphthalamine) 95%

In this study, five derivatives of Pyrimidine – 2 (1*H*) - one was prepared out of the reaction between one mole of both benzil and amine. The resulting compounds were reacted with acetone to prepare chalcone. The chalcones compounds were reacted with urea to synthesize five derivatives of imine – based 5,6 – dihydropyrimidin – 2(1*H*) – one.

the synthesized compounds were tested for their antibacterial activity against (*Staphylococcus aureus* and *Escherichia coli*). The compounds showed moderate effectiveness in inhibiting bacteria.

Synthesis of Imines. (0,014 mol) of benzil was dissolved in (30 mL) of absolute ethanol. Such solution was added to another solution prepared by dissolving(0,014 mol) of amine in (30 mL) of absolute ethanol. Such solution was stirred for 30 minutes and then refluxed at 78 ^{o} C away from light. TLC was used to know the end of the reaction. After the reaction was over, the product was filtered and recrystallized by the use of absolute ethanol [15], [16].

Synthesis of α, β-unsaturated Ketone. (0,014 mol) of the compound prepared in the above step was dissolved in (20 mL) of ethanol. (40 mol) of acetone was added thereto. The mixture was stirred and then a solution composed of (5 mL) of NaOH dissolved in (30 mL) of H_2O and such mixture was continually stirred. TLC was used to know the end of the reaction under ambient temperature. The product was then filtered and recrystallized by the use of ethanol [17], [19].

Synthesis of Pyrimidin-2(1*H*)-One Derivatives. (0,01 mol) of the α, β-unsaturated Ketone compound, prepared by the above reaction, was dissolved in (20 mL) of ethanol. A solution of (0,01 mol) of urea compound dissolved in (30 mL) of H_2O and 3 drops of glacial acetic acid were added thereto. The compound was refluxed under 80 ^{o} C. TLC was used to know the end of the reaction under ambient temperature. The product was then recrystallized by the use of ethanol [20], [23].

The equations for preparing the compounds are shown in Scheme 1, and the figures from 1 to 5 belong to the FTIR spectra. Figures from 6 to 10 belong to the ^{1}H NMR spectra while figures from 11 to 15 belong to the ^{13}C NMR spectra. Figures from 16 to 20 belong to the MASS spectra while the figure 21 shows the antibacterial activity. Table 1 lists the physical measurements of the prepared compounds. Table 2 lists the bacteria growth Inhabitation Diameter.

3. Results and Discussion

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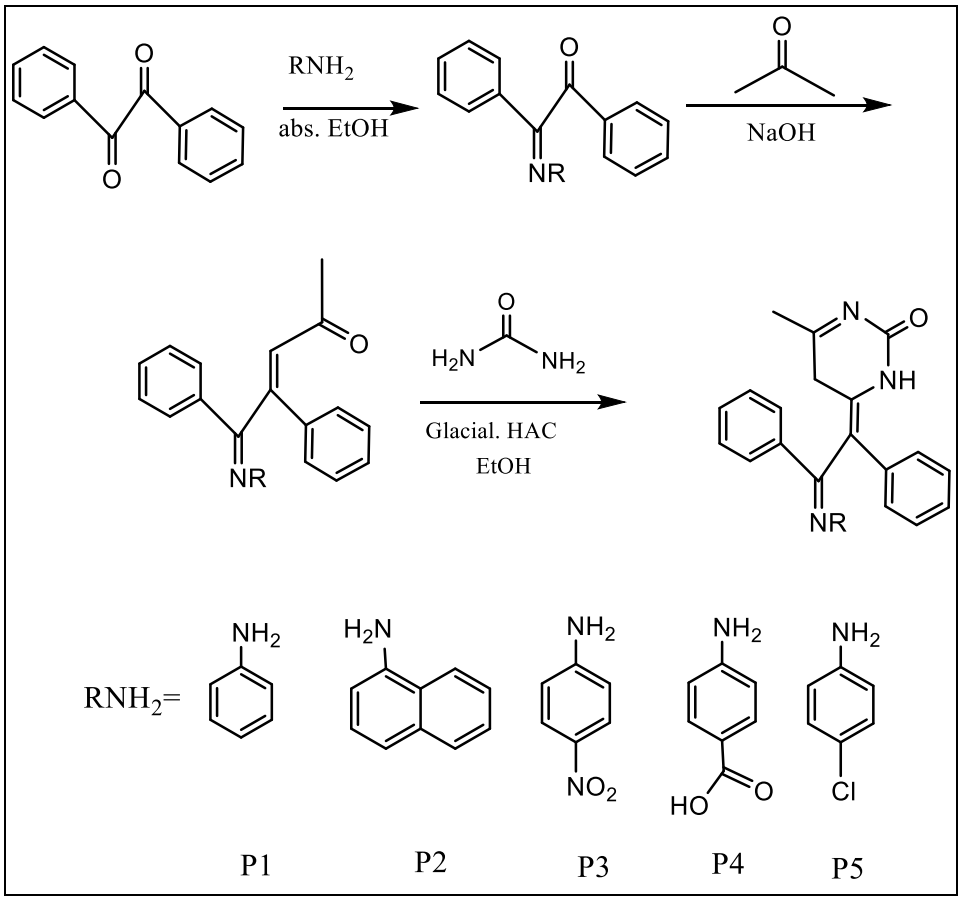

Scheme No. 1 The equations of synthesis 5,6-dihydropyrimidin-2(1*H*)-one associated with imine group.

Table No. 1 Properties of compounds 5,6-dihydropyrimidin-2(1*H*)-one Associated.

Compounds	Melting point ° C	Yield (%)	Color
P1	152-155	77	Orange
P2	132-134	77	Purple
P3	131-132	70	Yellow
P4	123-124	68	White
P5	130-133	72	Light red

The figures (1-5) below show the infrared spectra of compounds P1-P5.

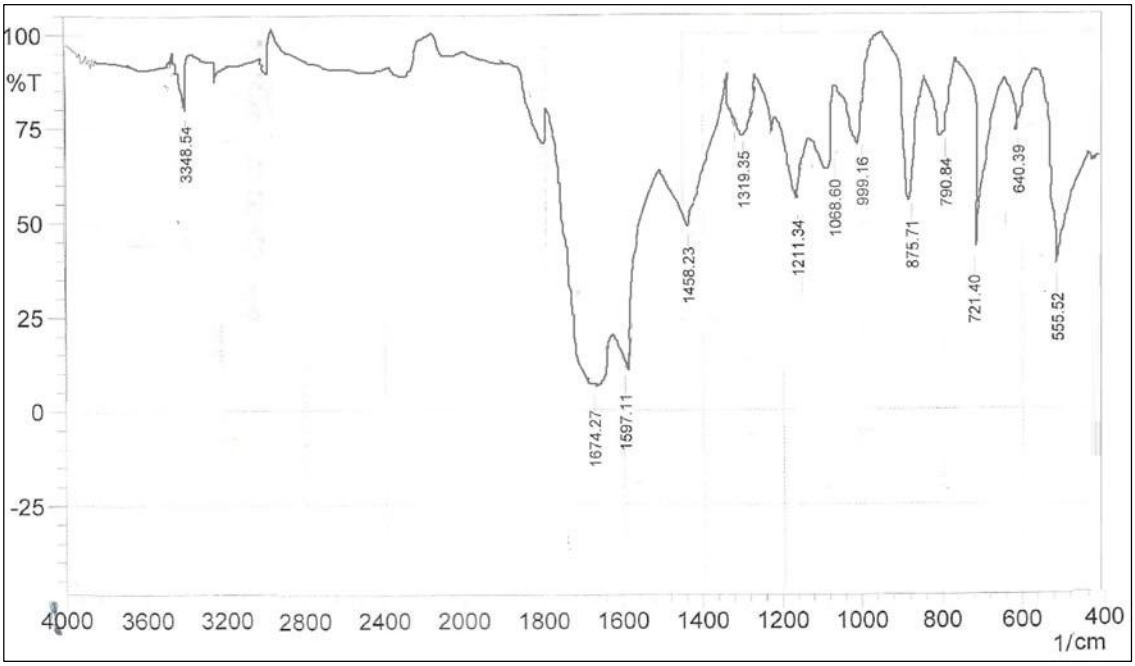

Figure No. 1 FTIR spectrum of compound P1

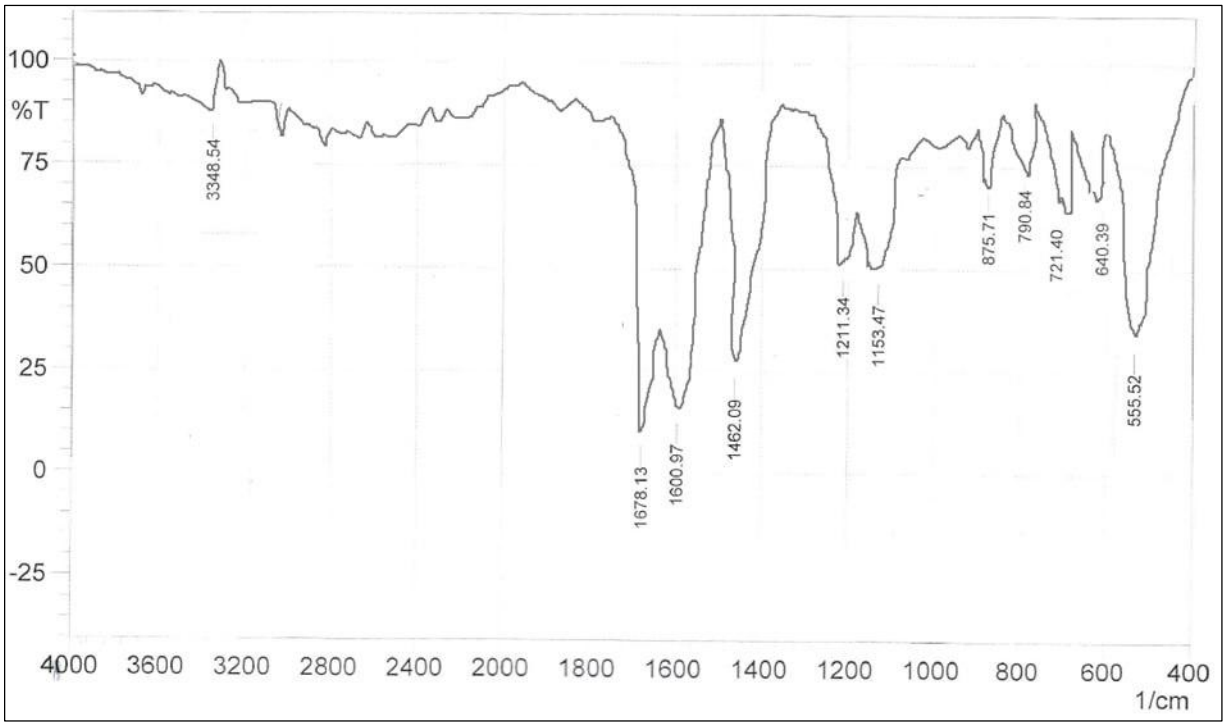

Figure No. 2 FTIR spectrum of compound P2

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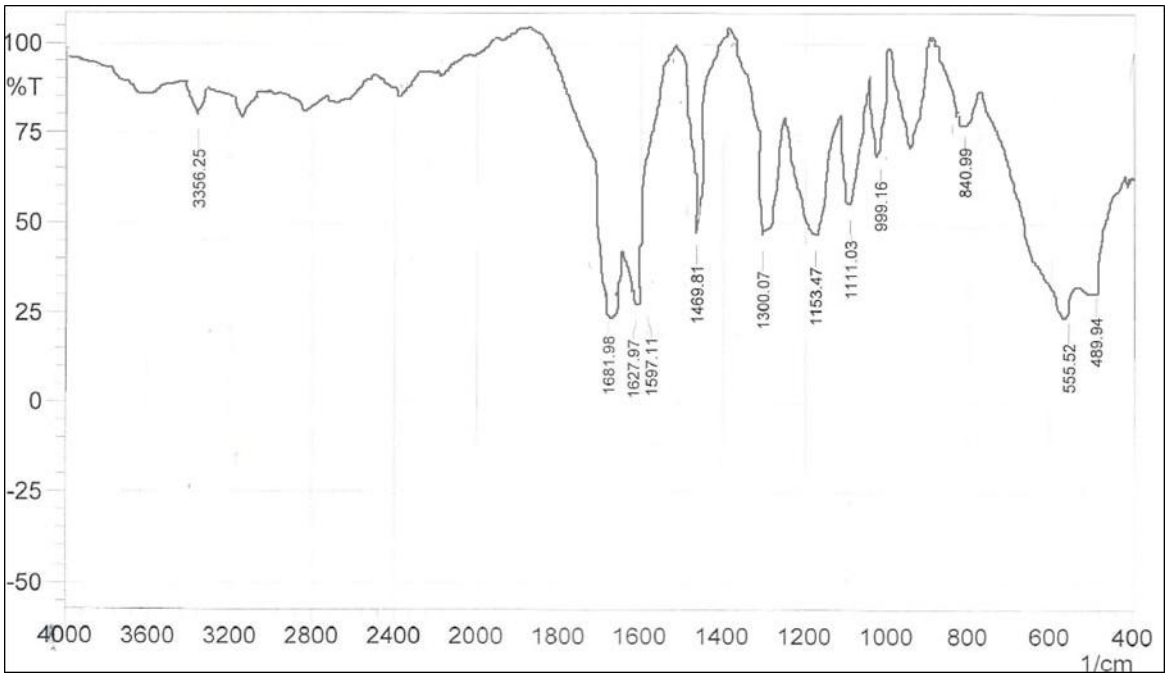

Figure No. 3 FTIR spectrum of compound P3

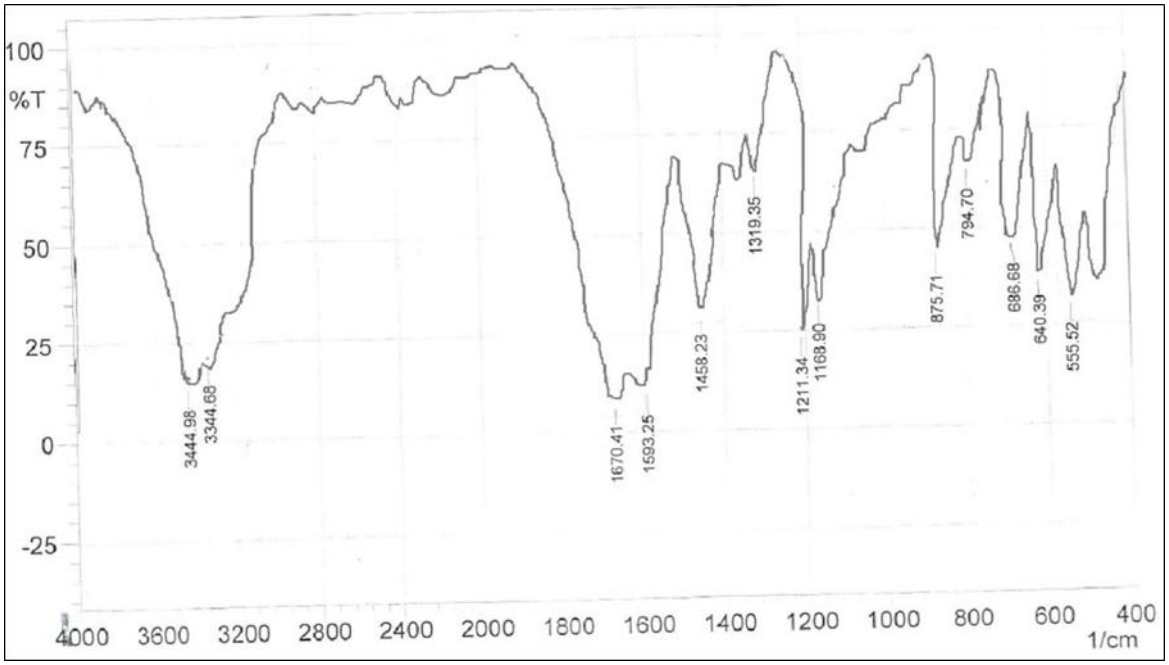

Figure No. 4 FTIR spectrum for compound P4

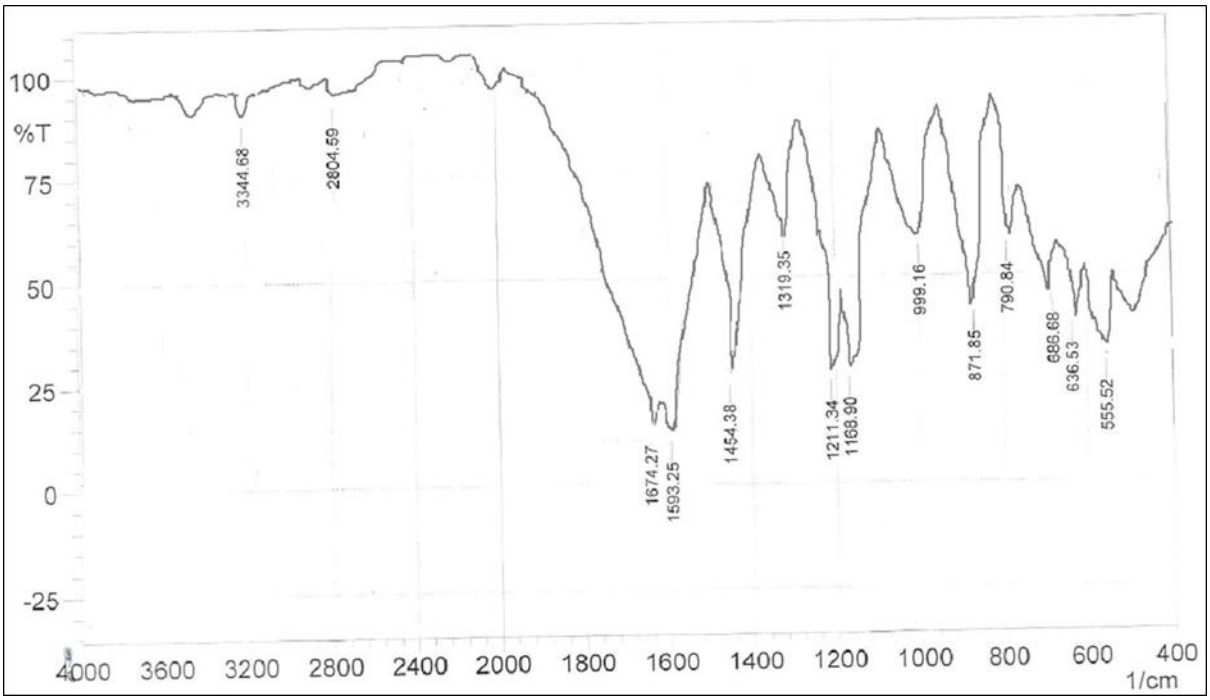

Figure No. 5 FTIR spectrum for compound P5

All of the above infrared spectra of the newly synthesized compounds showed the presence of a C=N bond stretching vibration in the imine group and the pyrimidine ring, as well as the presence of an N-H bond in the pyrimidine ring. The approved spectra also showed the presence of a C=O bond stretching vibration.

The figures (6-10) below show the ^{1}H NMR spectra of compounds P1-P5.

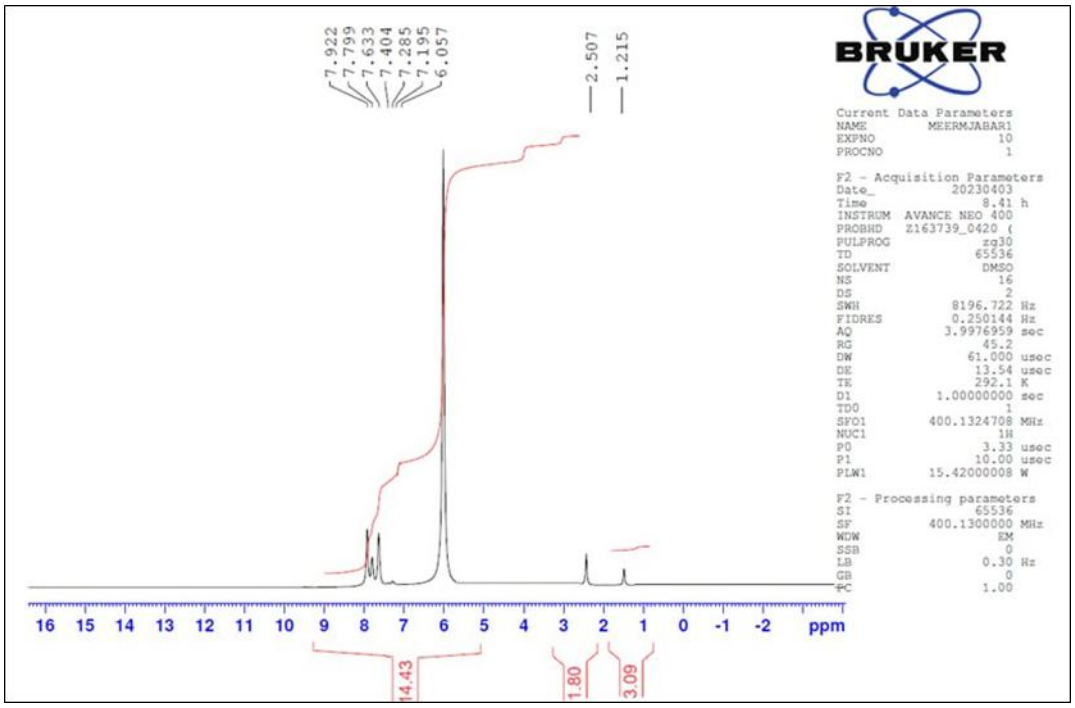

Figure No. 6 ^{1}H NMR spectrum of compound P1

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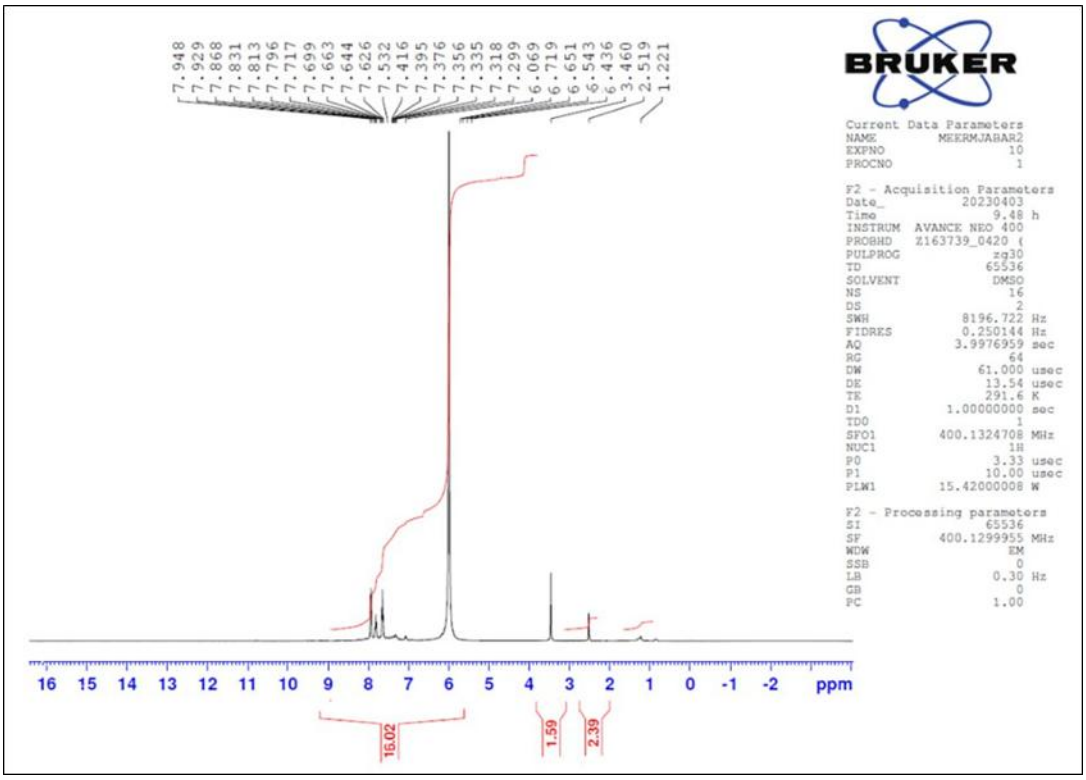

Figure No. 7 ^{1}H NMR spectrum of compound P2

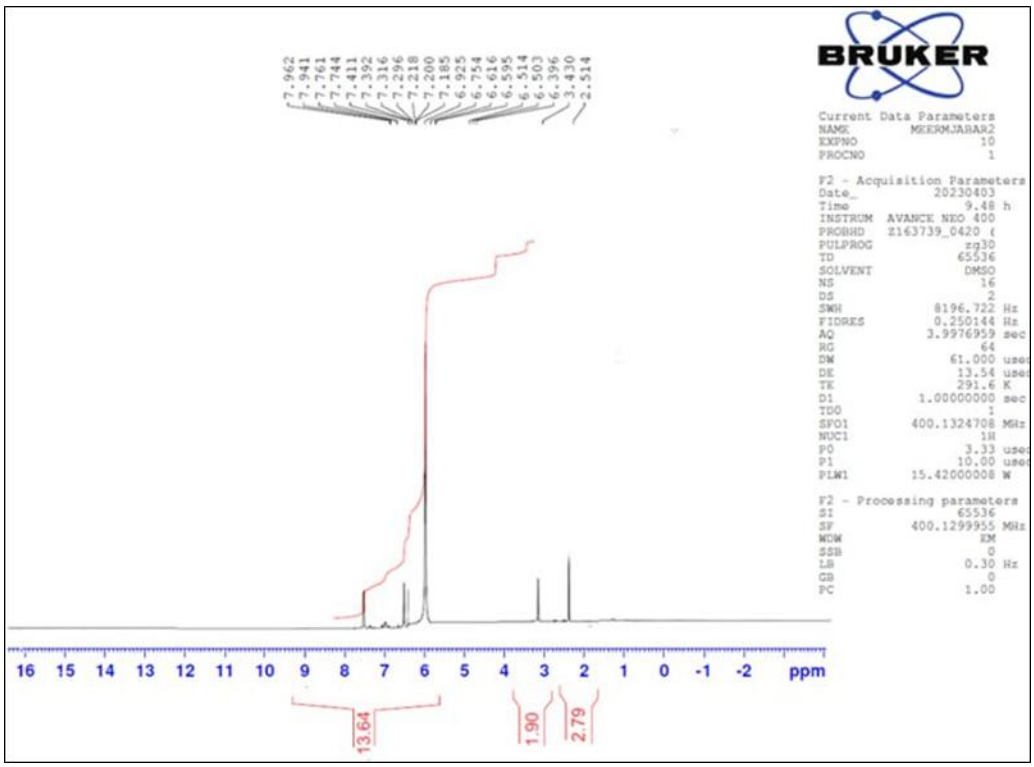

Figure No. 8 ^{1}H NMR spectrum of compound P3

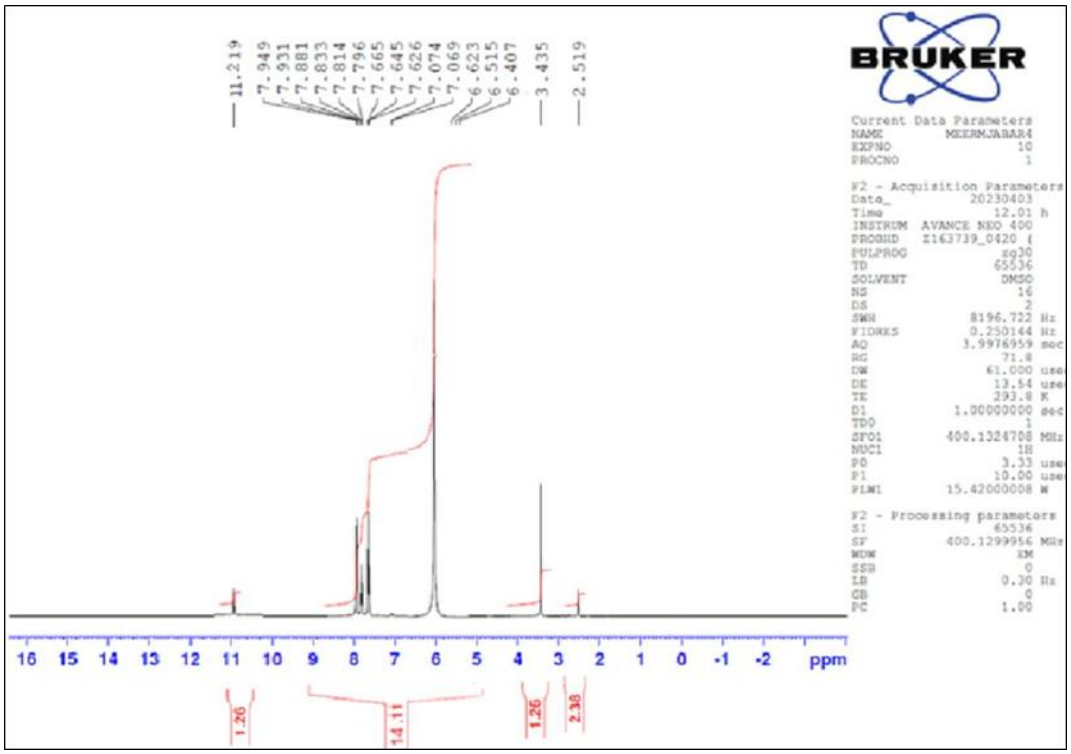

Figure No. 9 ^{1}H NMR spectrum of compound P4

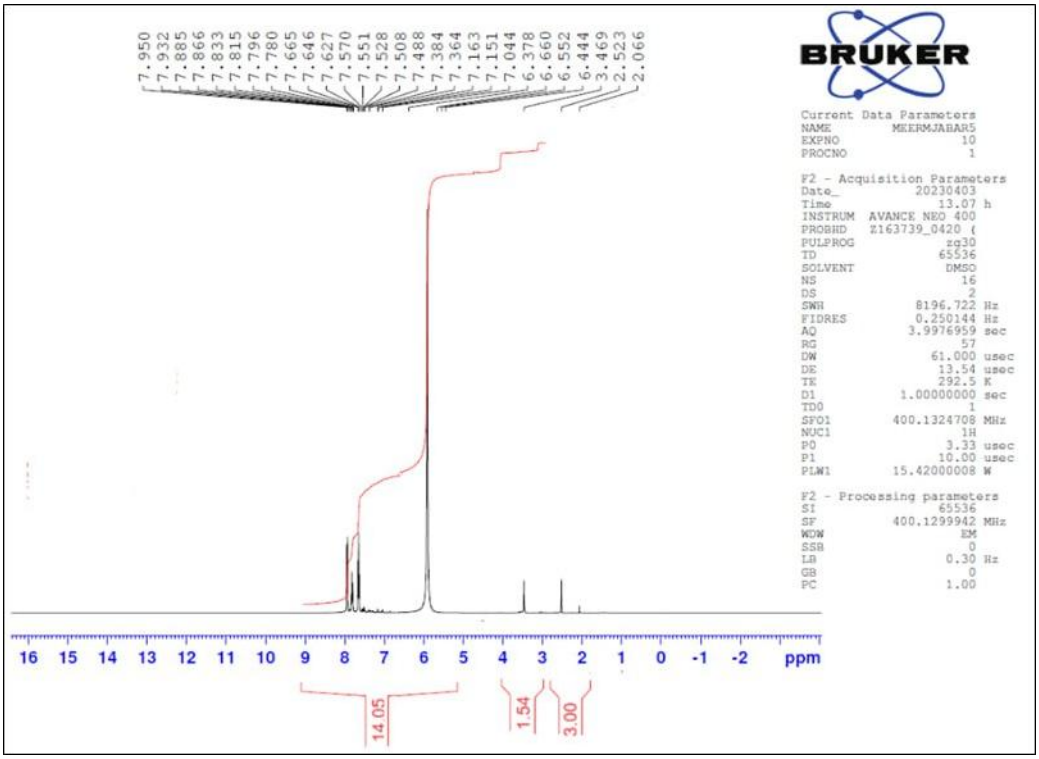

Figure No. 10 ^{1}H NMR spectrum of compound P5

All of the above proton NMR spectra of the prepared compounds confirmed the presence of a signal due to the proton of the NH group in the pyrimidine ring, as well as multiple signals due to the protons of the aromatic rings and the presence of a signal due to the three protons in the (CH_3) group.

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The figures (11-15) below show the ^{13} C NMR spectra of compounds P1-P5.

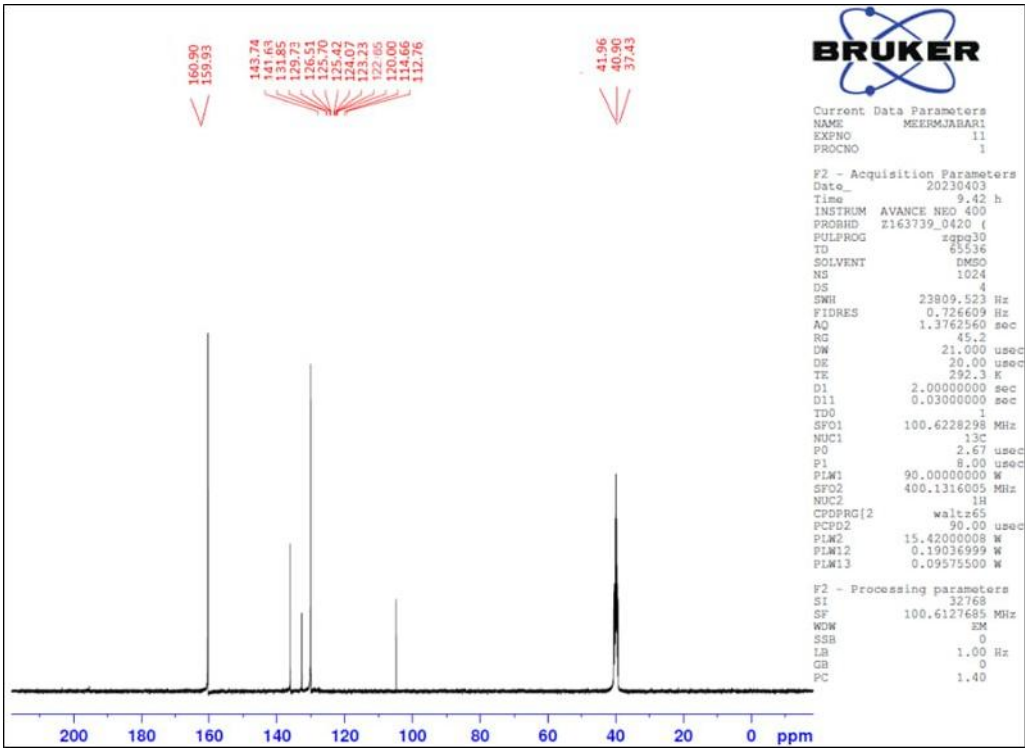

Figure No. 11 ^{13}C NMR spectrum of compound P1

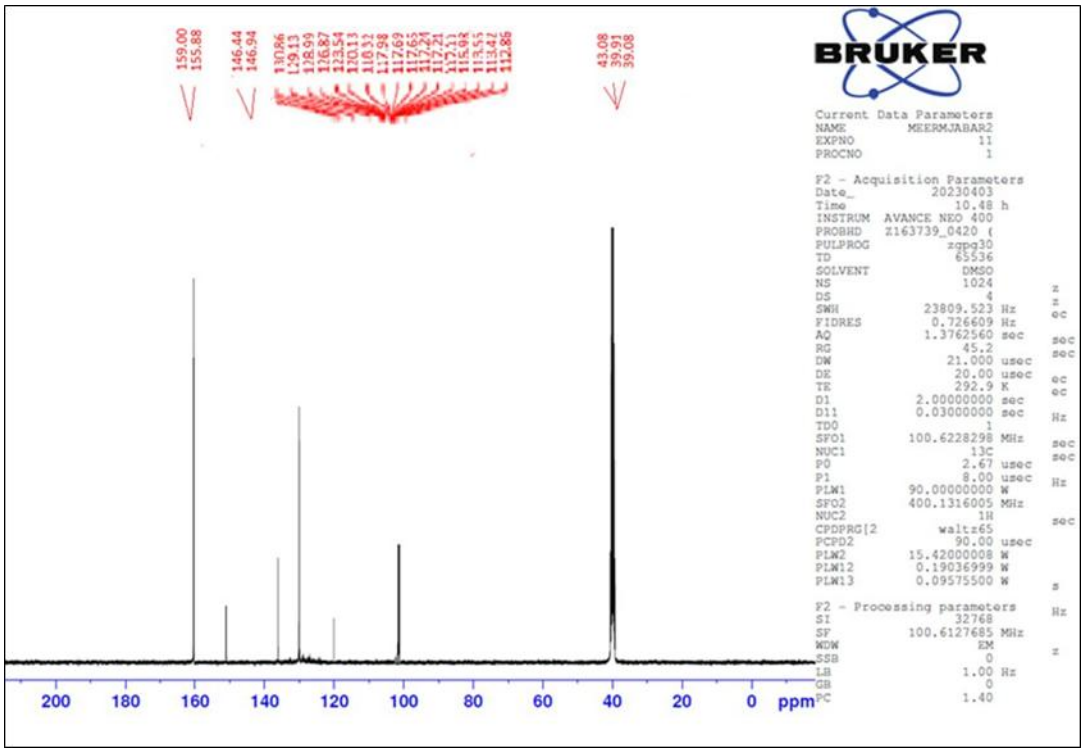

Figure No. 12 ^{13}C NMR spectrum of compound P2

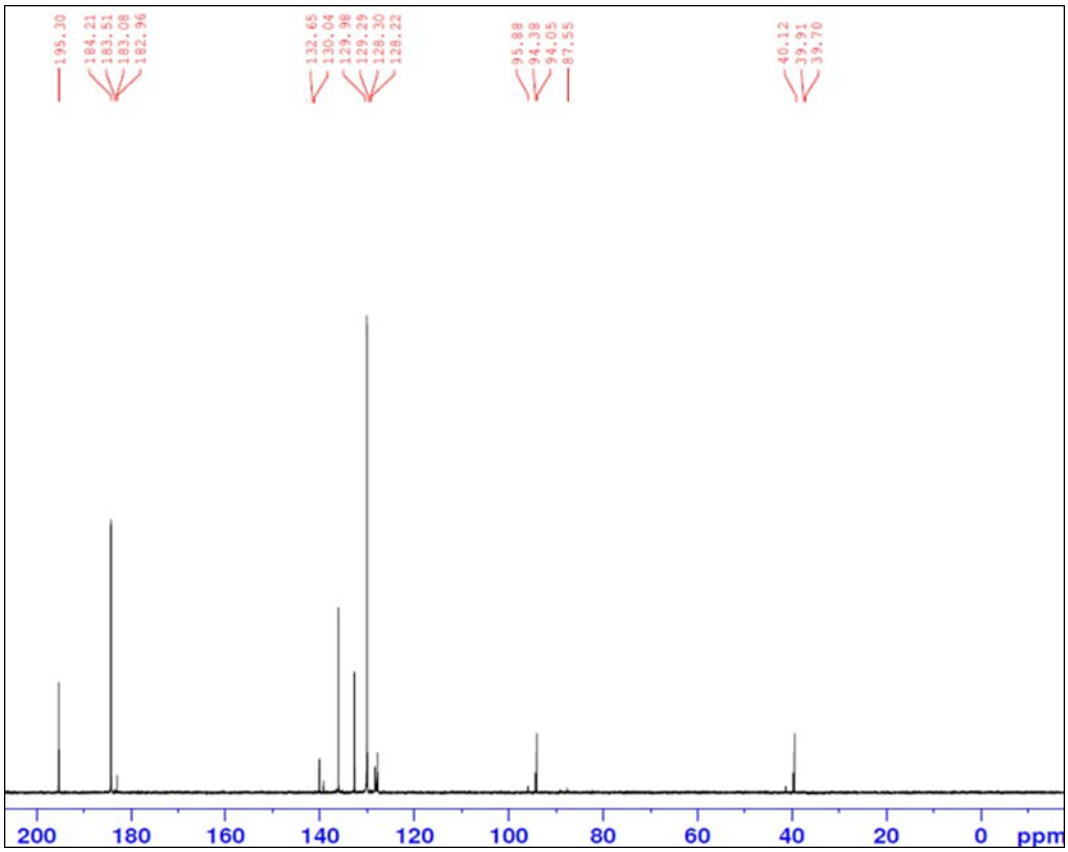

Figure No. 13 ^{13}C NMR spectrum of compound P3

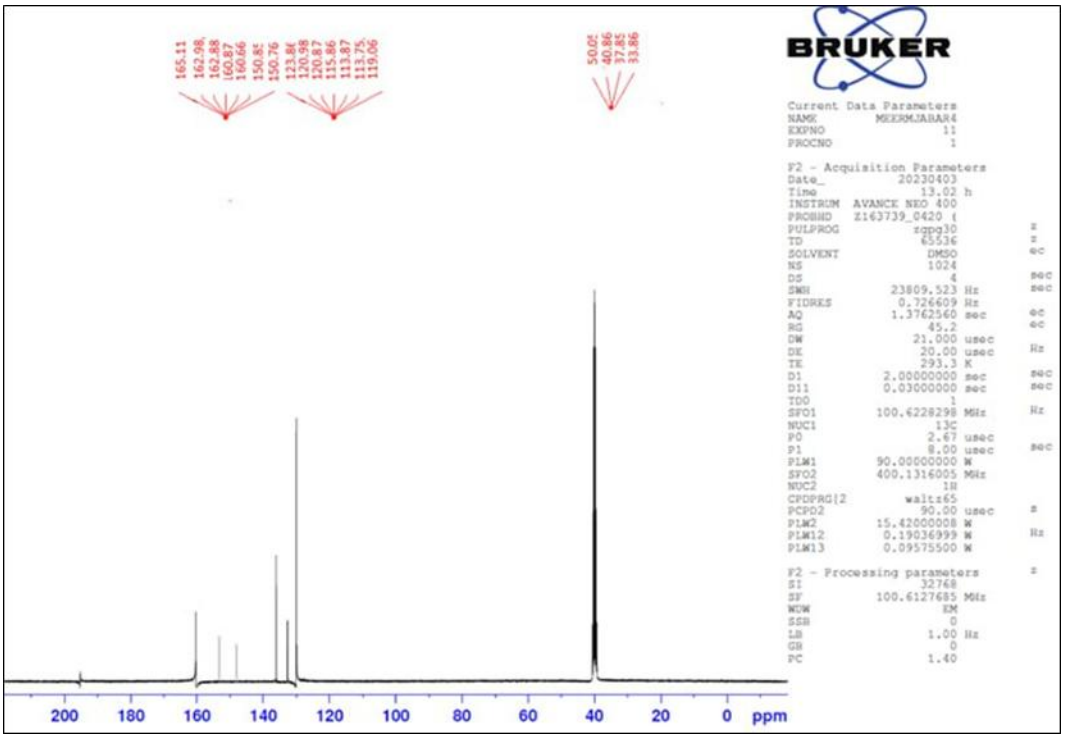

Figure No. 14^{13}C NMR spectrum of compound P4

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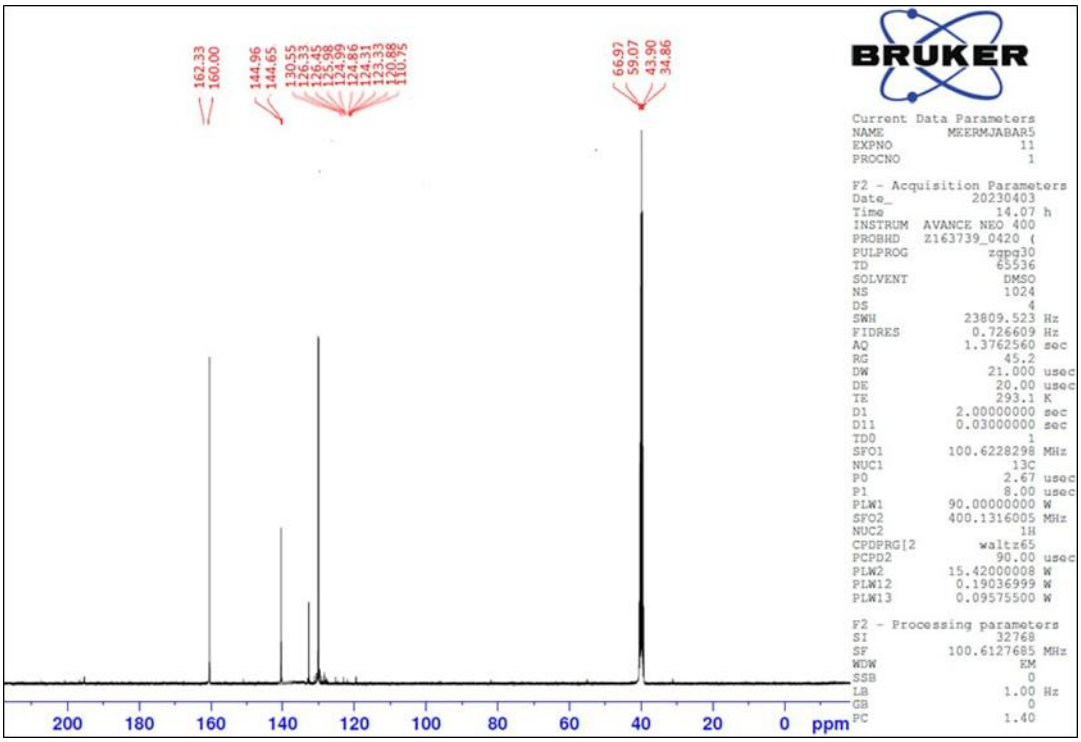

Figure No. 15 ^{13}C NMR spectrum of compound P5

All of the above NMR spectra of carbon-13 confirmed the predicted structures of the synthesized compounds, with a single signal appearing for the equivalent carbon atoms under electronic conditions. Different carbon atoms in the surrounding electronic conditions showed their own characteristic signal.

The figures (16-20) below show the MASS spectra of compounds P1-P5.

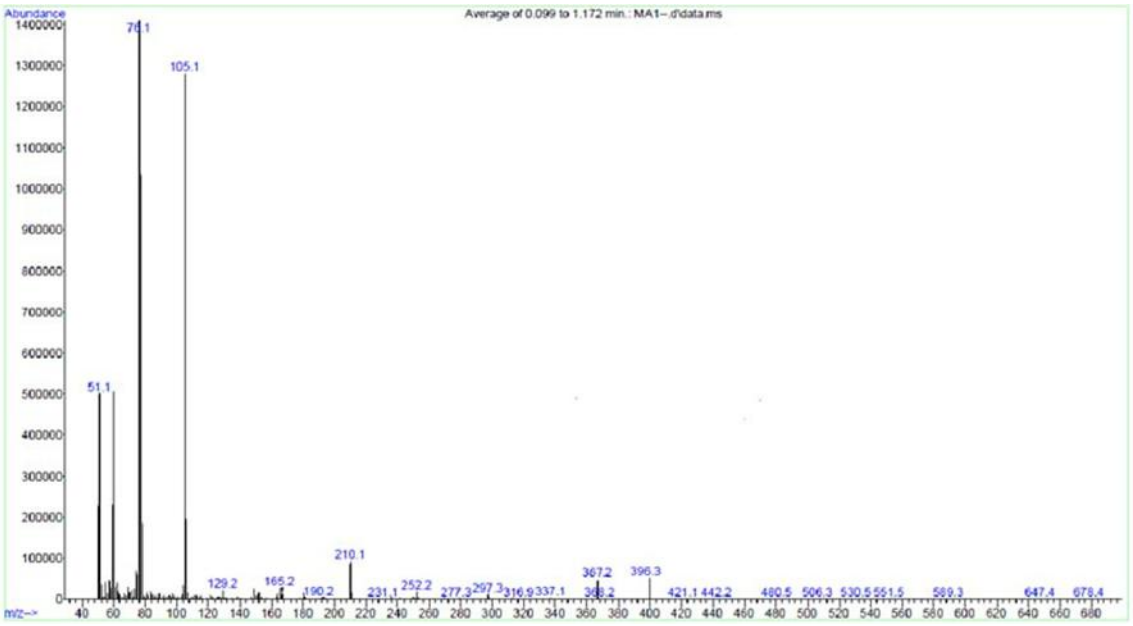

Figure No. 16 MASS spectrum of compound P1

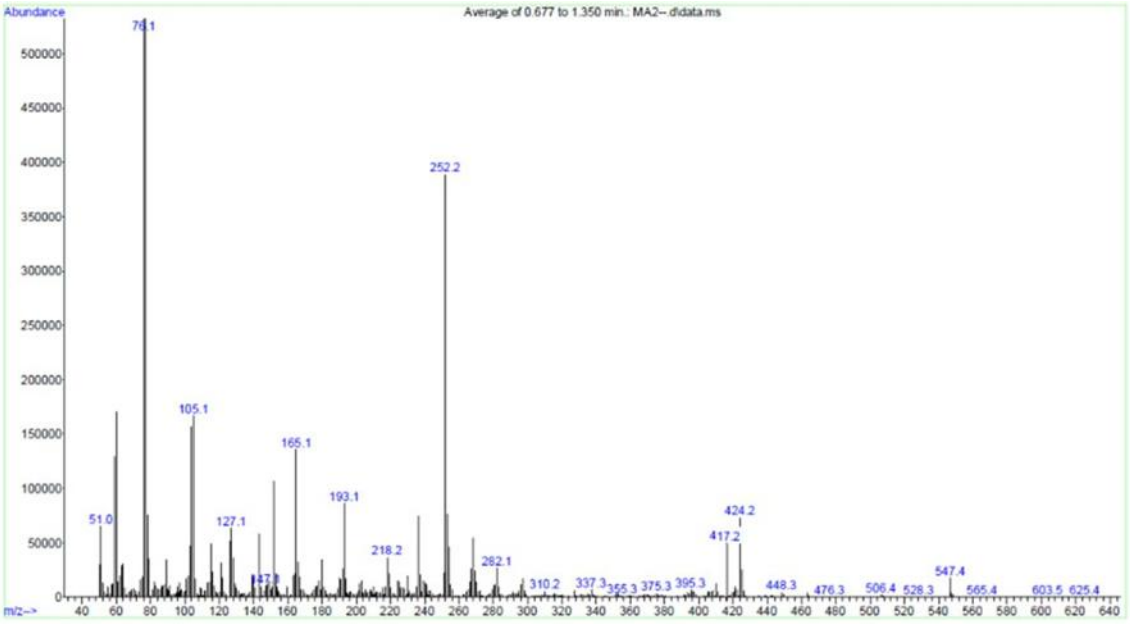

Figure No. 17 MASS spectrum of compound P2

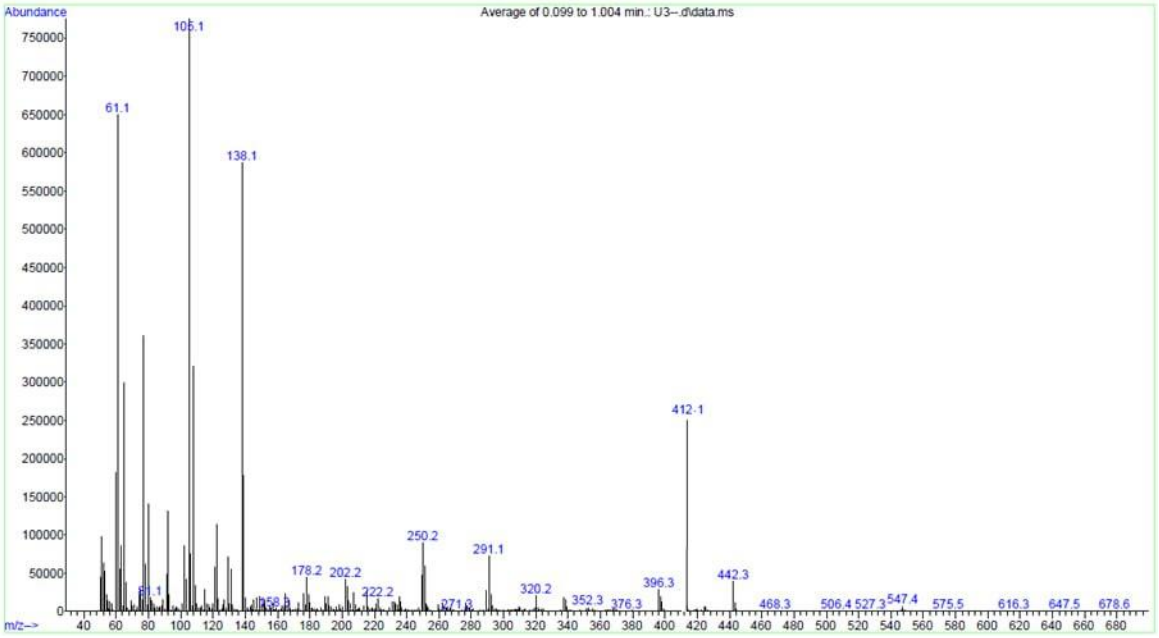

Figure No. 18 MASS spectrum of compound P3

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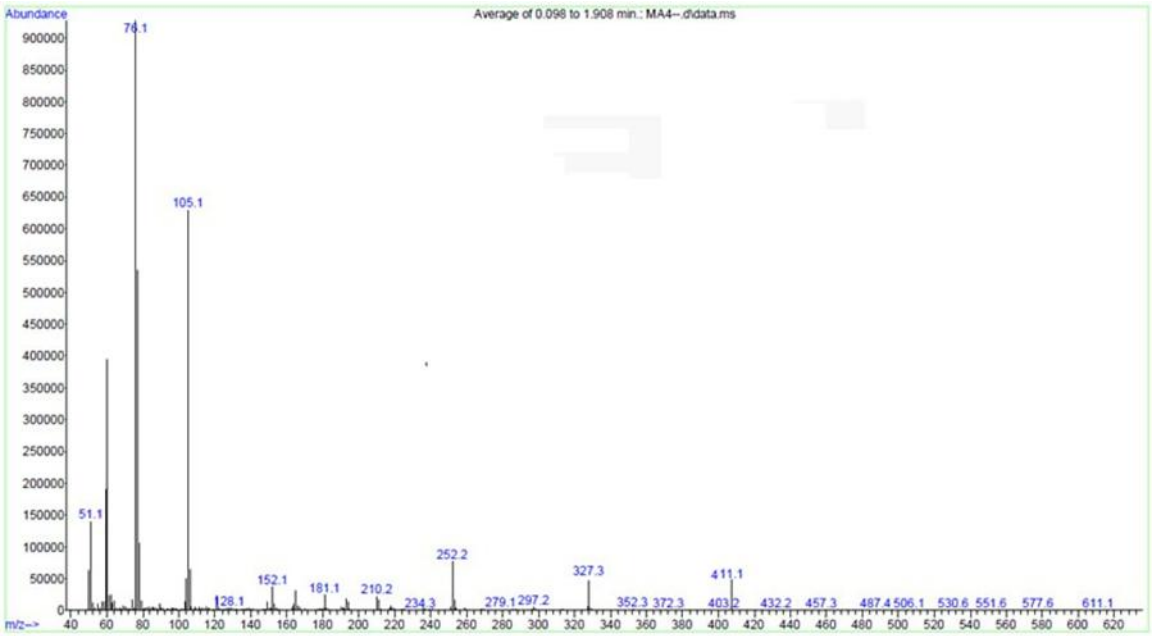

Figure No. 19 spectrum of compound P4

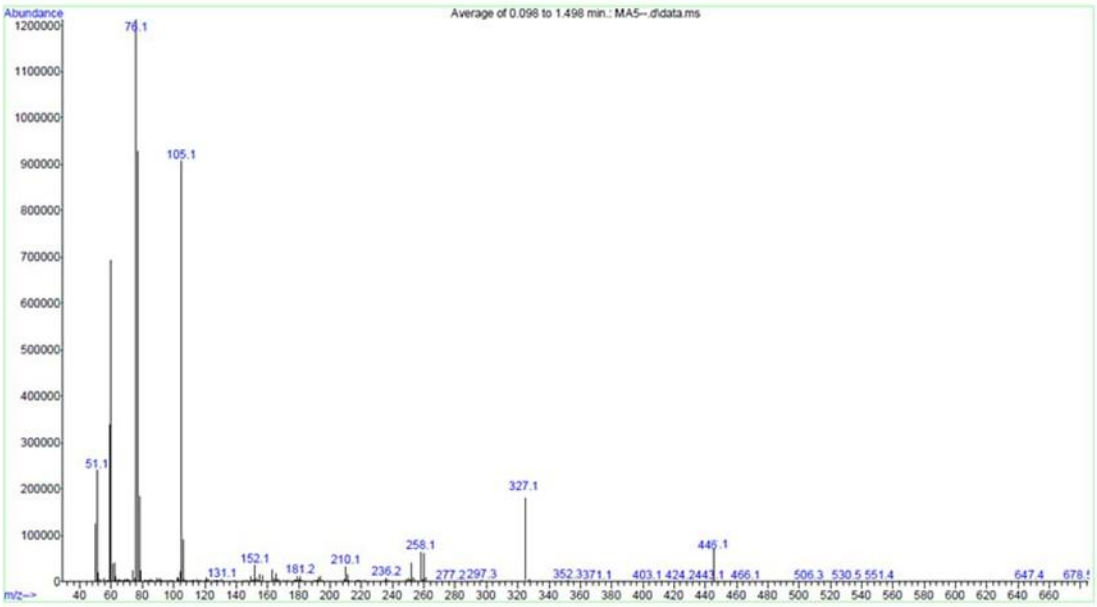

Figure No. 20 MASS spectrum of compound P5

All of the above MASS spectra showed the parent molecular ion and several other ionic fragments, which confirms the predicted structures of the newly prepared compounds.

(P1) 4-methyl-6-phenyl-6-(phenyl(phenylimino)methyl)-5,6-dihydropyrimidin-2(1*H*)-one.
Orange powder; Yield % 77; M. p, 152-155 ^{o} C; Infrared, *v* cm^{-1}: 1674 (C=O), 1624 (C=N), 1601(C=C), 2928-2799 (CH aliphatic), 3066 C-H) aromatic, 3344 (N-H); ^{1}H NMR, [CD_3Cl, 300 MHz, δ ppm]: 1.70 (S, 3H, CH_3), 7.61 (m, 5 H, Ph), 7.40 (m, 5 H, Ph), 7.65 (m, 5 H, Ph-N), 7.80 (s, H, NH), 1.26 (s, 2H, CH_2); ^{13} C NMR, [CD_3Cl, 300 MHz, δ ppm]: 37.43, 40.90, 41.96, 112.76, 114.66, 120.00, 12.86, 123.23, 124.07, 125.42, 125.70, 126.51, 129.73, 131.85, 141.63, 143.74, 159.93, 160.90. m/z, 367 (M^{+}).

(P2) 4-methyl-6-((naphthalen-1-ylimino) (phenyl)methyl)-6-phenyl-5,6-dihydropyrimidin-2(1*H*)-one.
Purple powder; Yield % 77; M. p, 132-134 ° C; Infrared, *v* cm^{-1}: 1669 (C=O), 1630 (C=N), 1610(C=C), 2933-2780 (CH aliphatic), 3101 C-H) aromatic, 3340 (N-H); ^{1}H NMR, [CD_3Cl, 300 MHz, δ ppm]: 2.51 (S, 3H, CH_3), 7.76 -7.12 (m, 13 H, Ar), 7.00 -6.87 (m, 4 H, Ar), 7.99 (s, H, NH), 3.20 (s, 2H, CH_2); ^{13} C NMR, [CD_3Cl, 300 MHz, δ ppm]: 39.08, 39.91, 43.08, 112.86, 113.42, 113.55, 115.98, 117.11, 117.21, 117.24, 117.65, 117.69, 117.98, 118.32, 120.13, 123.54, 126.87, 128.99, 129.13, 130.86, 146.94, 146.44, 155.88, 159.00. m/z, 417 (M^+).

(P3) 4-methyl-6-(((4-nitrophenyl) imino)(phenyl)methyl)-6-phenyl-5,6-dihydropyrimidin-2(1*H*)-one.
Yellow powder, Yield % 70; M. p, 131-132 ° C; Infrared, *v* cm^{-1}: 1609 (C=O), 1605 (C=N), 1597 (C=C), 2897-2806 (CH aliphatic), 3098 (C-H aromatic), 3365 (N-H), (s) 1390 - (as) 1520 (NO_2); ^{1}H NMR, [CD_3Cl, 300 MHz, δ ppm]: 1.22 (S, 3H, CH_3), 6.39-7.941 (m, aromatic proton), 7.96 (s, H, NH), 3.43 (s, 2H, CH_2); ^{13} C NMR, [CD_3Cl, 300 MHz, δ ppm]: 39.70, 39.91, 40.12, 87.55, 1294.05, 94.05, 94.38, 95.88, 128.22, 128.30, 129.29, 130.04, 132.65, 182.96, 183.08, 183.51, 184.21, 195.30; m/z, 412 (M^+).

(P4)4-(((6-methyl-2-oxo-4-phenyl-2,3,4,5-tetrahydropyrimidin-4-yl)(phenyl)(methylene)amino)benzoic acid.
White powder; Yield % 68; M. p, 123-124 ° C; Infrared, *v* cm^{-1}: 1666 (C=O), 1633 (C=N), 1616(C=C), 2977-2765 (CH aliphatic), 3113 C-H) aromatic, 3364 (N-H), 3500 (OH); ^{1}H NMR, [CD_3Cl, 300 MHz, δ ppm]: 1.30 (S, 3H, CH_3), 7.67 (m, 5 H, Ph), 7.43(m, 5 H, Ph),7.11 (m, 4 H, Ph-N), 7.91 (s, H, NH), 1.24 (s, 2H, CH_2), 11.80 (s, H, OH); ^{13} C NMR, [CD_3Cl, 300 MHz, δ ppm]: 33.86, 37.85, 40.86, 50.05, 119.06, 113.75, 113.87, 115.86, 120.87, 120.98, 123.86, 150.76, 150.85, 160.66, 160.87. 162.88, 162.98, 165.11, 165.32. m/z, 411 (M^+).

(P5) 6-(((4-chloroophenyl) imino) (phenyl)methyl)-4-methyl-6-phenyl-5,6-dihydropyrimidin-2(1*H*)-one.
Light red powder; Yield % 72; M. p, 130-133 ° C; Infrared, *v* cm^{-1}: 1654 (C=O), 1613 (C=N), 1600(C=C), 2873-2845 (CH aliphatic), 3100 C-H) aromatic, 3335 (N-H), 654 (C-Cl); ^{1}H NMR, [CD_3Cl, 300 MHz, δ ppm]: 1.13 (S, 3H, CH_3), 7.76 (m, 5 H, Ph), 7.54 (m, 5 H, Ph), 7.61 (m, 4 H, Ph-N), 8.10 (s, H, NH), 1.22 (s, 2H, CH_2); ^{13} C NMR, [CD_3Cl, 300 MHz, δ ppm]: 34.86, 43.90, 59.07, 66.97, 110.76, 120.88, 123.33, 124.31, 124.86, 124.99, 125.98, 126.45, 126.33, 130.55, 144.65, 144.96, 160.00, 162.33. m/z, 446 (M^+).

Five compounds were prepared out of Amine – based 5,6 – dihydropyrimidin – 2(1*H*) – one and such prepared compounds were traced by using spectrometry methods. All IR spectra of the prepared compounds have approved the presence of the stretching vibration of both (C=N) bond in the area (1633 – 1605) cm^{-1} and (N-H) bond in the area (3365 – 3335) cm^{-1}. Moreover, the approved spectra demonstrated the presence of a stretching vibration of the (C=O) group in the area (1674-1609) cm^{-1} while the stretching vibrations of (C-H) Aliphatic bond and (C-H) Aromatic bond were present in the areas (2933-2765) cm^{-1} and (3113-3066) cm^{-1}, respectively. The stretching vibration of the (C=C) bond occurred in the area (1600-1616) cm^{-1}. (P3) compound had two stretching vibrational bands belonging to the (NO_2) group in the area (s)1390-(as)1520 cm^{-1}. (P4) compound had a wide stretching vibrational band in the area (3500) cm^{-1} belonging to (OH) group while (P5) spectrum showed the presence of the prevailing stretching vibrational of (C-Cl) group in the area (654) cm^{-1}.
All ^{1}H NMR spectra of the prepared compounds confirmed the presence of a Multi-spectral signal in the area (7,77 – 7,61) ppm belonging to Aromatic protons and the presence of a signal in the area (1,70 – 1,13) ppm belonging to the protons of (CH_3) group.
The spectra also showed that the presence of a signal occurred in the area (1.64-1.20) ppm belonging to (CH_2) group while a signal belonging to (NH) group occurred in the area (8.10-7.80) ppm. The spectrum of

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(P4) compound showed a signal in the area (10.53) ppm belonging to (OH) group. All ^{13}C NMR spectra confirm the validity of the expected compositions of the prepared compounds. Mass spectra gave the mother ion and many other fragments which confirm the validity of the expected compositions.

The antibacterial study

The activity of the five compounds prepared in this study was screened by way of screening their antimicrobial activity. Such screening was conducted by the use of the diffusion method against two bacterial strains (*Staphylococcus aureus* and *E. coli*). Regarding such method, say, well-variant diffusion method, the used solvent was Dimethyl sulfoxide where an ultimate dissolving was obtained for the compounds prepared by the use of such solvent [24].

Bacteria Selection

The activity effect of the five compounds prepared in this study by way of overnight cultures against two types of bacteria, Staphylococcus aureus and Escherichia coli. The incubation period was 24 hours. The bacterial suspension was then diluted by the use of a Sterile Physiological solution in order to test the diffusion to 108 CFU / ML [25]. where:

(Turbidity = McFarland barium sulfate standard 0.5)

Agar diffusion well–variant

A piece of sterile cotton was used to spread the bacterial vaccine on a sterile Petri dish. The spread was made uniformly. The agar jelly contains 7 mm –-diameter holes containing spaces with 7 mm length. 50 microliter of the compounds which have been already prepared out of the compounds prepared with a concentration of 100 mg/mL. The dishes were then incubated at 36 o C ± 1o C for 24 hours with the presence of air. After the incubation period was over, the growth of bacteria was observed. Bacteria growth Inhabitation Diameter was measured in mm [26], [27].

The diameters of inhibition are listed in Table 2, while the images of inhibition of compounds are shown in figure 21.

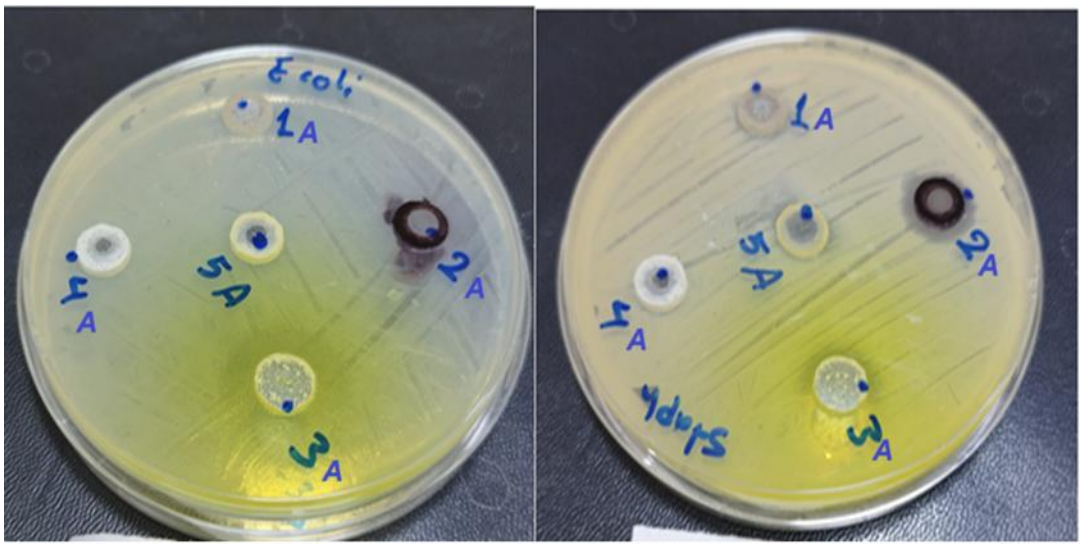

Figure No. 21 Photographic images of bacterial inhibition zones.

Table No. 2 Bacteria growth Inhabitation Diameter (mm).

Compounds	**Code**	**Concentration mg/mL**	**Inhibition zone(mm)** ***Staphylococcus aureus***	**Inhibition zone(mm)** ***E-coli***

P1	A1	100	10	zero
P2	A2	100	15	zero
P3	A3	100	12	20
P4	A4	100	zero	zero
P5	A5	100	zero	zero

The solvent dimethyl sulfoxide (CH_3CSCH_3) was used because it dissolves organic compounds well. To ensure that this solvent did not affect bacterial growth, it was used at a concentration of less than 1%. Table 2 indicates that the synthesized compounds were found to have a significant effect against bacteria (*Staphylococcus aureus*) in compounds P1, P2, and P3 only, and no effect on P4 and P5. The effect of compounds against (*E. coli*), only P3 was found to have an effect while the other compounds, P1, P2, P4, and P5, had no effect against this type of bacteria.

Regarding the antibacterial activity of the compounds against Staphylococcus aureus bacteria, compound P2 showed the highest activity against this type of bacteria. This may be due to the naphthyl group. The inhibition diameter was 15 mm. This group was able to penetrate the bacterial cell wall and bind to the target inside the cell. It is followed by compound P3 in inhibition, as the inhibition diameter reached 12 mm. The activity of this compound is attributed to the presence of the nitro group, which can easily bind to the target inside the cell because it is a charged group. As for compound P1, the inhibition diameter reached 10 mm. The ability of this compound is attributed to the presence of the phenyl group, which enables the compound to dissolve easily in the cell's lipid wall and enter the cell to bind to the target.

As for the compounds P4 and P5, they may not have the ability to penetrate the fatty cell wall because they are not able to dissolve in fats.

As for the effectiveness of the compounds against E. coli bacteria, it was found that compound P3 was the only compound that had effectiveness against this type of bacteria, and it was highly effective, and the diameter of inhibition was 20 mm. This is attributed to the presence of the NO2 group, which has the ability to penetrate the bacterial cell wall and bind to the target inside the cell. As for the other compounds, namely P1, P2, P4, and P5, their effectiveness was zero and they had no effect against bacteria. This may be attributed to the fact that the spatial shape of these compounds either does not help them dissolve in the cell wall or may not help them bind to the target inside the cell.

4. Conclusion

In this study, five derivatives of 5,6 dihydroprimind – 2 (1*H*) – one compound were synthesized by reacting 1 mole of benzil with 1 mole of five types of Amine. The resulted was converted into Chalcone by the reaction with Acetone and then the final compound was prepared by reaction of chalcone with Urea. The validity of the prepared compounds was confirmed by spectroscopy methods (MASS, IR and NMR).

The antibacterial activity of the synthesized compound against two types of bacteria (Staphylococcus aureus and Escherichia coli) was also studied and accordingly it has been approved that such prepared compounds do have a moderate anti-bacterial activity. It was found that one compound (P3) has an inhibitory effect on the bacteria *E. coli*, as the diameter of inhibition was 20 mm. As for the bacteria *Staphylococcus aureus*, compounds P1, P2, and P3 had an inhibitory effect, and the best of them was compound P2, as the diameter of inhibition was 15 mm.

5. Conflict of interest

There is no conflict of interest.

6. References

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